Cyclin-dependent kinase 4 (Cdk4) partners with D-type cyclins (CycD) to drive the cell cycle and proliferation. Selective Cdk4 ATP-site inhibitors such as the breast cancer drug palbociclib are showing remarkable success in the clinic, so it is critical to understand mechanisms controlling Cdk4 function and how they impact inhibitor activity. The p27 protein is known as a negative regulator of proliferation, primarily through its inhibition of Cdks in G1, but p27 also plays an important role in assembling Cdk4-CycD complexes. We have found that p27, when phosphorylated by tyrosine kinases, allosterically activates Cdk4-CycD1. Our structural and biochemical data reveal that p27 remodels the kinase ATP site to phosphorylate the retinoblastoma tumor suppressor protein (Rb) and other substrates. Surprisingly, purified p27-Cdk4-CycD1 complexes and endogenous Cdk4 complexes are insensitive to inhibition by palbociclib. We find that palbociclib inhibits Cdk4-p27 association and sequesters monomer Cdk4 in breast tumor cells. Our data redefine p27 as both a Cdk4 activator and inhibitor and implicate prevention of active enzyme assembly as a direct target of Cdk4 chemical inhibitors.